THE EFFECT OF LOW TEMPERATURES ON HYDRA.

CAROLINE McGILL,

INSTRUCTOR IN ANATOMY, UNIVERSITY OF MISSOURI.

In his experiments on *Hydra*, Greely, '03, observed that when the temperature is reduced, there occurs, what he termed, a reversal of vital phenomena. The body is quickly reduced to a resting stage, to an undifferentiated mass of protoplasm. He describes the changes as follows: "Whenever a *Hydra* is exposed to a temperature of 4° to 6° C., the tentacles gradually become shorter and thicker, and are finally completely absorbed into the body. As the absorption goes on, the ectoderm and endoderm cells of the tentacles lose their individuality and form an undifferentiated mass of protoplasm which is slowly taken into the body of the *Hydra*. The tentacleless body of the *Hydra* becomes slowly resolved into a dense spherical mass of coagulated protoplasm, in which no distinction between the individual cells can be made out, and remains in this condition as long as it is kept at a low temperature, but quickly forms tentacles and a double layer of cells again when it is returned to the temperature of the room. If *Hydra* in the earlier stages of the process of budding be placed at a temperature of 4° C., not only does the growth of the bud stop instantly but absorption of the bud into the body of the parent commences, and continues until all traces of the bud have disappeared. . . . Six or seven days are required for the complete disappearance of the bud. . . . Lowering the temperature brings about a reversal of vital phenomena and the formation of simple resting stages."

Greely, '01–'02, in an earlier series of experiments traced in protozoa a similar reversal of vital phenomena brought about by reducing the temperature. He found that when the temperature is lowered the protoplasm of unicellular forms coagulates, with accompanying loss of water and the cells pass into resting stages. His experiments on *Hydra* led him to conclude that similar changes may take place in metazoa.

Reversal of vital phenomena due to causes other than reduction of temperature has also been described. Loeb, '00, in campanularian hydroids produced similar effects in the polyps by bringing them in contact with solid bodies. In the same paper he describes like changes brought about by gravity. In *Antennularia* he found that when the branches are placed horizontally the polyps on the lower side are quickly absorbed.

Miss Thacher, '03, working on *Eudendrium*, *Pennaria*, and *Campanularia*, repeated Loeb's experiments, studying at the same time the histological changes. She found that the absorption as described by Loeb is not due to contact with solid bodies, but that it is a true degeneration caused by unfavorable conditions. At all times whether in contact with foreign bodies or not she found that the polyps of these hydroids are absorbed when brought into the laboratory and that the absorption is always preceded by degeneration.

Gast and Godlewski, '03, describe a similar degeneration of the polyps of *Pennaria* when kept in the laboratory.

The series of experiments described in this paper was begun with a view only of determining the histological changes which take place in *Hydra* when the temperature is lowered. Greely in his work considered merely the grosser structural changes. It was soon found that *Hydra* when subjected to low temperatures, often, in fact, usually do not behave as described by Greely. Exposure to a temperature of 2° C. for nine days, that is two degrees lower and three days longer than he found necessary to obtain a complete resting stage, if other conditions such as the composition of the water in which the *Hydra* are kept are unchanged. produces little or no effect on the structure. There may be no indication whatever of a reversal of vital phenomena. Because of this direct contradiction of results, it was thought advisable to repeat Greely's experiments, using as large a number of *Hydra* as possible.

Hydra fusca and *Hydra viridis* were the forms used. *Hydra fusca* is more favorable for experimental work than is *Hydra viridis*, since it is much more resistant to changes in the composition of the water; and also to sudden changes in the temperature. However with proper care either *Hydra* may be employed.

During the experiments the *Hydra* were usually kept in water from the ponds where they had been collected, although they appear to live as well in ordinary tap water. The water was changed frequently since even at low temperatures there is considerable evaporation.

When the *Hydra* were to be studied histologically they were fixed in an extended condition in a hot corrosive-acetic mixture, run up through the graded alcohols, embedded in paraffin and cut in sections, 5γ thick. The sections were stained in hæmatoxylin-eosin.

The series includes about twenty experiments on something like seventy-five *Hydra*. From this number, five typical experiments have been chosen for description. Two of these were on *Hydra* collected in the winter, three on those collected in the summer.

Experiment 1. — February, 1907. One well-expanded brown *Hydra* was placed in an ice box, kept at a temperature of 2° C. The *Hydra* slowly contracted. At the end of nine days it was removed and appeared then as shown in Text-fig. 1, *B*. The body and tentacles had contracted until they were about one third the normal expanded length. The body had decreased somewhat in volume as if shrinkage had taken place. Upon removal from the ice box the temperature of the water around the *Hydra* was quickly raised to that of the room. When the temperature reached 8° C., the *Hydra* began to expand and by the time it had reached 10°, the *Hydra* had stretched to the length shown in Text-fig. 1, *C*. It had just the appearance of a normal expanded *Hydra* except that the tentacles were slightly shorter and somewhat opaque. When stimulated the *Hydra* rapidly contracted to the size shown in Text-fig. 1, *A*. Viewed under the low power of the microscope, while still alive it showed apparently normal structure; two distinct cell layers, nematocysts, etc. Ten minutes after its removal from the ice box the *Hydra* was fixed. Sections of the fixed *Hydra* showed practically normal cell structure, Fig. 6. The *Hydra* used in this experiment was taken from a pond having a temperature of about 10° C.

Experiment 2. — November, 1906. A brown *Hydra* with a small bud was kept at a temperature of 4° to 6° C. for eight days.

(The *Hydra* had been collected in a pond having a temperature of 12° C.) When removed from the ice box both body and tentacles were about one half contracted. The bud was about the same size it was when the experiment was begun. Sections showed body, tentacles and bud all with distinct cell structure, Figs. 1–4. The minute changes in structure shown by these sec-

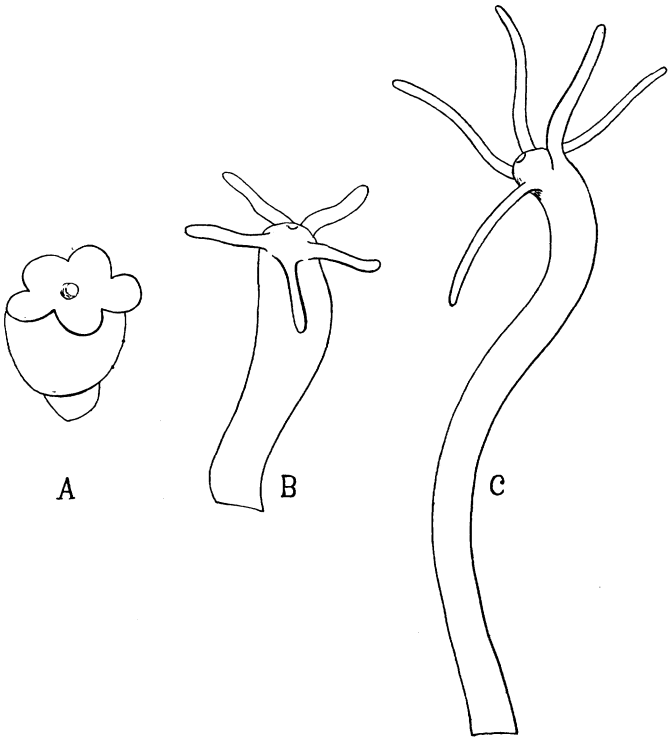

FIG. 1. *Hydra* after an exposure of nine days to a temperature of 2° C.; *B*, when taken from the ice box; *C*, two minutes later, the *Hydra* now at the temperature of the room; *A*, one minute later still; the expanded *Hydra* had been stimulated and rapidly contracted into this small mass.

tions will be described in detail in a separate paragraph farther on.

Experiment 3. — July, 1907. A brown *Hydra*, taken from a pond having a temperature of 30° C. was kept at 4° for six days. It gradually contracted and when removed appeared as drawn in Text-fig. 2, *A*. The body was a spherical mass with tentacles almost completely withdrawn. As the temperature was raised it

began to lengthen but could not stretch out to more than one third the length of the normal expanded *Hydra*. The *Hydra* was fixed and sectioned. The sections show distinct cells but in places, especially in the tips of the tentacles, there are marked cytological changes, changes such as might be brought about by a rapid loss of water.

Experiment 4. — July, 1907. The *Hydra* used in this experiment was collected at the same time as that used in experiment 3. It was a large brown *Hydra* with two buds, one large with long tentacles the other small with no tentacles. It was kept at a temperature of 4° to 6° C. for six days. At the end of the experi-

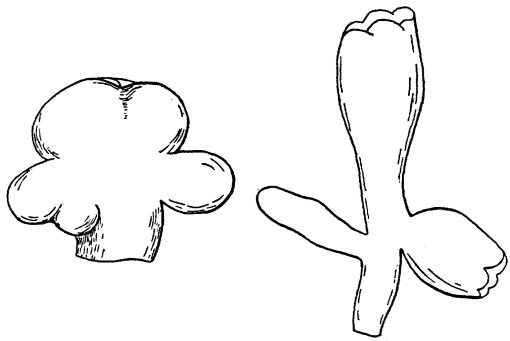

FIG. 2. A budding *Hydra* after an exposure of six days to a temperature of 4°; *A*, when taken from the ice box; *B*, two minutes later when brought to the temperature of the room.

ment it was firmly contracted, Text-fig. 2, *A*. When the temperature was raised it rapidly became active and in a few minutes extended until it appeared as shown in Text-fig. 2, *B*. It seemed incapable of expanding farther. The *Hydra* was fixed and sectioned. It still had clear cell structure, Fig. 5.

Experiment 5. — July, 1907. A *Hydra* taken from a pond at a temperature of 30° C. was kept for seven days at 4°. At the end of the time it was contracted into an oval mass, the tentacles showing only as tiny knobs. When brought to the room temperature it could expand only very slightly, so little, in fact, that some magnification was necessary in order to detect it. When observed under the microscope while still alive no cell layers could be seen. Sections of the *Hydra* after fixation,

in only a few places showed cell boundaries. The body was in the main a mass of protoplasm with degenerating nuclei scattered through it, that is a syncytium. The nuclei were small and pycnotic, showing direct evidence of degeneration.

From the above experiments it will be seen that the changes which take place in *Hydra* when subjected to low temperatures, are somewhat variable. Those collected in summer, when the water in which they have been living is warm react differently from those collected in winter when the water is cold. In *Hydra* from cold ponds (temperature 8° to 12° C.) lowering the temperature to 2° and keeping it there for as long as two weeks has little effect except to cause contraction for the time being. As soon as the temperature is raised, they assume their ordinary expanded form. Usually the tentacles are slightly altered. They become opaque at the tips and cannot expand as far as before exposure to cold. *Hydra* that are budding show no absorption of the bud such as described by Greely. As soon as such *Hydra* are placed at room temperature the buds, as well as the parent body, become actively contractile.

Hydra collected in summer when the pond water is warm show more marked effects when the temperature is reduced. They contract into small masses and when brought, after several days exposure to cold, into water the temperature of the room, do not immediately expand to their normal length. Even in summer *Hydra* I have never observed absorption of the buds except in cases where there was distinct degeneration, due to some other condition than low temperature. Both summer and winter *Hydra*, when the temperature is reduced, lose in volume. This loss is more pronounced in the summer than in the winter *Hydra*.

The cytological changes brought about by low temperatures are interesting. They are in the main such as can be ascribed to loss of water. When more marked changes take place they are always accompanied by distinct evidences of degeneration. The temperature effects are always much more pronounced in *Hydra* collected in summer than those collected in winter. Fig. 6 is drawn from the endoderm of a winter *Hydra* which had been kept at a temperature of 2° C. for nine days. The structure is

almost identically that of a normal *Hydra*, except that the vacuoles in the cytoplasm are not quite so large and the nutrient spheres are not so numerous. The distended condition of the gland cells is worthy of note. This *Hydra* has practically the same structure throughout that one deprived entirely of food for an equal length of time would have. Here, then it is impossible to say whether there are any changes whatever due directly to reduction of temperature. In this *Hydra* there are, at any rate, no indications of Greely's reversal of vital phenomena. Figs. 1–4 are from sections of a winter *Hydra* after being kept eight days at a temperature of 4° to 6° C. Fig. 1 shows the entire thickness of the wall of the tentacle. The protoplasm of both endoderm and ectoderm cells is less vacuolated than in the normal *Hydra* and the nuclei, especially those of the interstitial cells are smaller and more deeply staining. In Fig. 2 the differentiation of the endoderm of the body into endoderm cells and gland cells is apparent. Even in the bud of this *Hydra* the cells are still intact. Fig. 4 shows a foot cell still filled with secretion. The sections of this *Hydra* show no changes that might not be directly due to loss of water.

In a *Hydra* collected in summer and placed at a temperature of 4° to 6° C. for six days the cytological changes are much more pronounced than in the two winter *Hydra* just described (see Fig. 5). Here the cells are much smaller than normal, the protoplasm is free from vacuoles, gland cells cannot be distinguished in the endoderm, and the nuclei of all the cells are very small and deeply staining. Many of the nuclei, especially those of the interstitial cells, look as if the nuclear sap had been almost entirely extracted leaving only the much condensed chromatin within. In a few places in this *Hydra*, most apparent at the tip of the tentacles, the cell boundaries are indistinct. Where such is the case the nuclei always are beginning to degenerate.

In *Hydra* like the one described in experiment 5, where, after exposure to cold, the body practically loses power of movement even after the temperature is raised, the cell structure is often entirely obliterated. The nuclei are in such cases always pycnotic and both nuclei and cytoplasm show every evidence of degeneration. Since it is seldom that *Hydra* are found which behave in

this manner when the temperature is lowered, it is probable that the loss of cell boundaries is due to some other condition than change of temperature. An entirely similar effect may be produced by keeping the *Hydra* in water that is not frequently renewed. It is possible that the reversal of vital phenomena described by Greely may have been merely a degeneration brought about by some such unfavorable condition. That he was able to get such *Hydra* to return to normal after bringing them to the room temperature for several days is no indication that they had been only in a resting condition. If after the temperature was raised the adverse conditions were at the same time removed, then any intact cells which may have escaped degeneration, might quickly regenerate the entire body. The ability of *Hydra* to regenerate from a few cells is well known.

Greely's statement that *Hydra* when kept at low temperature are resolved into undifferentiated protoplasm is misleading. It is impossible to tell whether he meant merely that the cell boundaries are destroyed, the nuclei remaining intact; or whether the nuclei too, are broken down so that the body is made up of a simple protoplasmic mass entirely devoid of differentiation. The former is probably what he meant. In such event it is possible that after all cell boundaries are destroyed regeneration may take place, but in this series of experiments no evidence of this has been seen. It is most likely that in all his *Hydra* that passed from his so-called resting stage to a normal condition, there had been only a partial destruction of cells, and that the few remaining cells regenerated the body.

Though Greely's results were not corroborated by these experiments yet his statement that reduction of temperature does bring about a loss of water is substantiated. All the effects due to lowering the temperature I think can be ascribed to this cause.

In concluding, it may be repeated that reduction of temperature for the length of time mentioned by Greely does not cause *Hydra* to be resolved into undifferentiated protoplasm. When this does take place it is due to unfavorable conditions and is a degeneration effect and not a temperature effect.

This work was done in the Zoölogical Laboratory of the University of Missouri under the direction of Professor Lefevre, to whom I am indebted for many valuable suggestions.

LITERATURE.

1. **Greely.**
'01 On the Analogy between the Effects of Loss of Water and Lowering the Temperature. The American Journal of Physiology, Vol. VI.

2. **Greely.**
'02 The Artifical Production of Spores in Monas by a Reduction of the Temperature. Biological Bulletin, Vol. III.

3. **Greely.**
'03 Further Studies on the Effects of Variations in the Temperature on Animal Tissues. Scientific Papers.

4. **Gast and Godlewski.**
'03 Ueber den Regulations bei Pennaria carolinii. Arch. fur Entw. der Organismus, Bd. XVI.

5. **Loeb.**
'00 Transformation and Regeneration of Organs. American Journal of Physiology, Vol. IV.

6. **Thacher.**
'03 Absorption of the Hydranths in the Hydroid Polyps. Biological Bulletin, Vol. III.

Explanation of Plate IV.

Abbreviations. *En*, endoderm cell; *ec*, ectoderm cell; *l*, supporting lamella; *ic*, interstitial cell; *nut*, nutrient sphere; *nem*, nematocyst; *s gl*, secretion granule; *gl*, gland cell of the endoderm; *p*, pigment; *cnid*, cnidoblast cell.

Fig. 1. Cross-section of the tentacle of a *Hydra* exposed eight days to a temperature of 4° to 6° C. *En*, endoderm; *nut*, nutrient sphere; *l*, supporting lamella; *ic*, interstitial cell; *ec*, ectoderm cell; *nem*, nematocyst.

Fig. 2. Endoderm from the body of the same *Hydra* as Fig. 1. *En*, endoderm cell; *gl*, gland cell; *p*, pigment; *nut*, nutrient sphere.

Fig. 3. Cross-section of the body of the bud of the same *Hydra* as Fig. 1. *En*, endoderm; *l*, supporting lamella; *ec*, ectoderm.

Fig. 4. Gland cell from the foot of the *Hydra* described in Fig. 1. *S g*, secretion granules.

Fig. 5. Cross-section of the body wall of a *Hydra* exposed for six days to a temperature of 4° to 6° C. This *Hydra* had been collected in summer from a warm pond. Notice the few vacuoles in the cytoplasm. *P*, pigment; *en*, endoderm; *l*, supporting lamella; *cnid*, cnidoblast cell; *nem*, nematocyst; *i c*, interstitial cells.

Fig. 6. Endoderm cells from the body wall of a *Hydra* which had been exposed to a temperature of 2° C. for nine days. The structure is almost identically that of a normal *Hydra*. *Nut*, nutrient sphere; *gl*, gland cell; *p*, pigment.

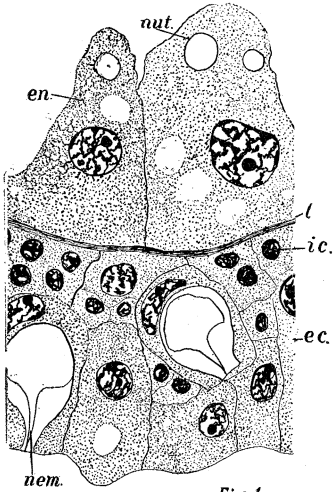

Fig. 1

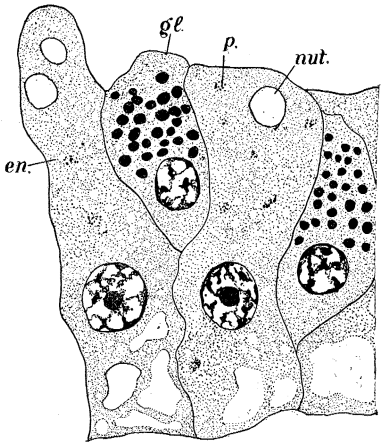

Fig. 2

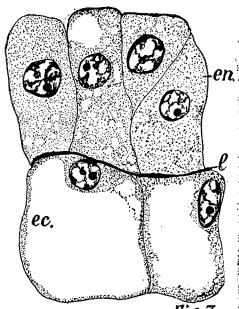

Fig. 3

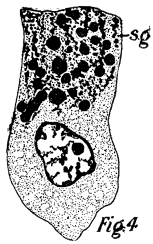

Fig. 4

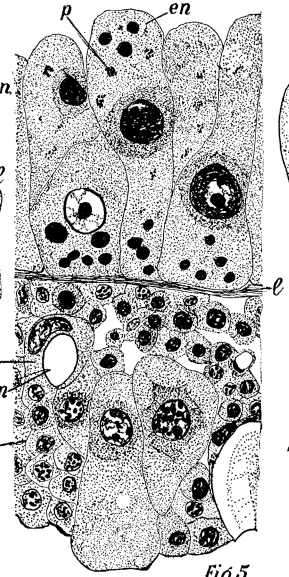

Fig. 5

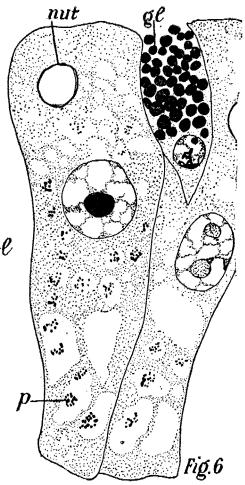

Fig. 6